様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Molecular dynamics of the fusion oncoprotein EML4-ALK in lung cancer as revealed by high-speed AFM

研究課題名（英文）Molecular dynamics of the fusion oncoprotein EML4-ALK in lung cancer as revealed by high-speed AFM

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研究成果の概要（和文）：EML4-ALKは強力な肺がんドライバー融合タンパク質であり、その構造－活性制御相関は腫瘍学・構造生物学において注目されている。ただし、EML4-ALK分子全体の構造は不明である。本研究では、分子動態計測可能な高速原子間力顕微鏡を用いて、EML4-ALKモノマー、ダイマー、トリマーの構造動態を解明することに成功した。興味深いことに、ALK阻害剤はIDPR領域の歪みを介してダイマー形成を抑制した。本研究によりEML4-ALKバリアント体それぞれの構造動態プロファイルが初めて明らかになるとともに、本研究はEML4-ALK阻害剤の作用機作解明や新たな阻害剤創成の基盤になると期待される。

研究成果の学術的意義や社会的意義
This is the first dataset to describe the overall structures of the fusion oncoprotein EML4-ALK variants by HS-AFM, which greatly broadens our knowledge of this oncoprotein. As EML4-ALK is an important therapeutic target, this work would be important in both academic and societal implications.

研究成果の概要（英文）：Fusion oncoprotein EML4-ALK is one of the strong drive mutations in lung cancer. However, the overall structure remains unclear because the N-terminal region is an intrinsically disordered protein region (IDPR) whose structure is hard detectable by conventional tools. In this study, we have imaged the real-time structures of EML4-ALK in monomer, dimer, and trimer forms by using a high-speed atomic force microscope (HS-AFM). Interestingly, we found the dimer is more stable than the trimer and the ALK inhibitors can indirectly suppress the dimeric rates through the IDPR region distortion. This work clarified the structural profiles of IDPR in EML4-ALK fusions for the first time, and our data suggested new strategies against the cancers of EML4-ALK based on the overall structures.

研究分野：Cancer biology

キーワード：ALK　EML4　HS-AFM　fusion oncoprotein　lung cancer　structure

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９、ＣＫ－１９（共通）

１．研究開始当初の背景

The genomic rearrangement between echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK) generates an EML4-ALK fusion driver oncoprotein found in 3-7% of patients with non-small cell lung cancer (NSCLC). EML4-ALK occurs in 15 variant isoforms due to the different breakpoints in the EML4 gene during the rearrangement. Variant-1 (v1) was defined as a full-length version, and variants-3 (v3) and-5 (v5) with different sizes of deletion in the EML4 were known as the typical variants. The N-terminal region of variant EML4-ALK is a structurally flexible 'Intrinsically Disordered Region' of which structure cannot be revealed by X-ray crystallographic and cryo-electron microscopic (Cryo-EM) approaches. Thus, whole structures of EML4-ALK have remained unknown.

Patients bearing the EML4-ALK are initially sensitive to ALK tyrosine kinase inhibitors (ALK-TKI) but would invariably develop substantial resistance. The structural variation of EML4-ALK has recently been proposed to participate in resistance to ALK-TKI. A new peptide designed to inhibit protein association by the TD domain can inhibit ALK-TK activity, suggesting the overall structure is also important. Thus, further understanding of the whole structures of EML4-ALK is significantly required for the next generation of drug design.

High-speed atomic force microscopy (HS-AFM) is a groundbreaking technology established by researchers in the WPI-Nano Life Science Institute at Kanazawa University. HS-AFM possesses the unique ability to investigate oligomer structures, real-time molecular flexibility, and changes in structures of proteins in native solution conditions, including proteins that have intrinsically disordered regions. Based on all these backgrounds, the application of HS-AFM to the structural analysis of EML4-ALK variants has advantages in the elucidation of unresolved issues as described above.

２．研究の目的

Taking advantage of HS-AFM technology, this project aims to elucidate unresolved issues that relate to entire structures and functional significance of EML4-ALK variants and mechanisms of drug resistance attributable to their structural changes. This project elucidates (1) whole structures of variants; (2) different abilities and involvement of domains in monomer↔dimer/trimer formation; (3) the relationship between resistance/ sensitivity and structural changes caused by ALK-TK inhibitor; (4) the mechanism of the structural changes with ALK inhibitors.

3.　研究の方法

To finish this project, we performed: (1) EML4-ALK proteins of v1, v3, and v5 expressions in mammalian cells and purification. This step included the EML4-ALK plasmid construction and the identification of the purified protein. (2) HS-AFM imaging: Analysis of the variant EML4-ALK proteins under the HS-AFM. Firstly, it requires an optimized condition to gain a clear structure of the monomeric protein and identification of the domains under the HS-AFM. Imaging analysis and quantification with statistical tools are critical points. (3) Dimer and trimer confirmation: In the same condition, we try to confirm whether the dimeric or trimeric forms are detectable or not and whether their structural difference exists or not. (4) Analyzing the mechanism of the structural difference and the affection by ALK inhibitors under HS-AFM, is the most challenging part of this project.

４．研究成果

(1) **Monomer structure of EML4-ALK:** Three typical EML4-ALK variant proteins have been purified for high-speed AFM imaging. We have firstly imaged the monomer of EML4-ALK v5, v3, and v1. To measure the two-dimensional (2D) end-to-end distance (R2D) of the IDRs, we followed the method of the previous study (Kodera, *et al.* Nanotech., 2019). Quantification data of the length distribution also suggested that v3 has a longer IDR than v5 and a shorter IDR region than v1, which is consistent with their different amino acid sequences. GFP was fused into the C-terminal of variant-5 as a control and the HS-AFM video suggested that GFP can form an additional smaller domain adjacent to the main globule and no affection to the IDR region. Thus, we have imaged the monomeric structure of Eml4-ALK.

(2) **Dimer and trimer of Eml4-ALK:** We have also imaged the dimer and trimer for all three variants in the same condition. Representative images suggested EML4-ALK variants can form dimerization with a shape of a cherry-like pattern and trimerization with a pattern of clover. Although the general patterns were very similar in all three variant EML4-ALK forms, variant-3 dimers and trimers displayed double and multiple Gaussian distributions of ALK height, and these patterns were not found in v1 and 5, suggesting v3 has a different profile of the dimeric and trimeric in the overall structures. Considering that v3 causes the most aggressive form of the disease, this result might be

significant on the clinic side.

(3) **Dynamic processes from monomer to trimer:** As the monomer, dimer, and trimer forms of EML4-ALK have been imaged, we asked how the dimer and trimer formation? Our data suggested that the EML4-ALK dimer initially interacted with the IDR region to build a bridge between the ALK domains, and then the two ALK domains gradually got close, and finial associated together. Based on these results, the association from both IDR and ALK terminals could generate a more stable dimer or trimer than the initial association.

(4) **ALK inhibitors decreased the dimeric rates:** As the dimer and trimer are important for the activation of EML4-ALK. A fundamental question is which is the main form of EML4-ALK in physiological conditions. To answer this question, we have investigated dimer and trimer rates in a large-scale observation for all three variants. Our results showed that the dimer is more stable than the trimer, and the v3 dimer has the highest dimer rate, larger than the v5 dimer rate and v1 dimer rate. These data pointed out the dimer rate is higher than the trimer rate in all variants of proteins.

In conclusion, we have completed the project to image the overall profiles of fusion oncoprotein EML4-ALK and gain a further understanding of the structural differences between the variant proteins. Our data may provide new clues to the mechanism of drug resistance.

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Wang Miaoxing、Han Xujun、Liu Chuyan、Takayama Rie、Yasugi Tetsuo、Ei Shin-Ichiro、Nagayama Masaharu、Tanaka Yoshitaro、Sato Makoto	４．巻 12
２．論文標題 Intracellular trafficking of Notch orchestrates temporal dynamics of Notch activity in the fly brain	５．発行年 2021年
３．雑誌名 Nature Communications	６．最初と最後の頁 1～14
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-021-22442-3	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Wang Rong、Yamada Tadaaki、..., Yano Seiji	４．巻 11
２．論文標題 Transient IGF-1R inhibition combined with osimertinib eradicates AXL-low expressing EGFR mutated lung cancer	５．発行年 2020年
３．雑誌名 Nature Communications	６．最初と最後の頁 1
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-020-18442-4	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Han Xujun、Wang Miaoxing、Liu Chuyan、Trush Olena、Takayama Rie、Akiyama Takaaki、Naito Toshiki、Tomomizu Takeshi、Imamura Kousuke、Sato Makoto	４．巻 33
２．論文標題 DWnt4 and DWnt10 Regulate Morphogenesis and Arrangement of Columnar Units via Fz2/PCP Signaling in the Drosophila Brain	５．発行年 2020年
３．雑誌名 Cell Reports	６．最初と最後の頁 108305～108305
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.celrep.2020.108305	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Liu Chuyan、Trush Olena、Han Xujun、Wang Miaoxing、Takayama Rie、Yasugi Tetsuo、Hayashi Takashi、Sato Makoto	４．巻 11
２．論文標題 Dscam1 establishes the columnar units through lineage-dependent repulsion between sister neurons in the fly brain	５．発行年 2020年
３．雑誌名 Nature Communications	６．最初と最後の頁 1
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41467-020-17931-w	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関